Potential Health Impact of Nanoparticles

Tian Xia,[1] Ning Li,[1,2,3] and Andre E. Nel[1,2,3,4]

[1]Division of NanoMedicine, Department of Medicine, University of California, Los Angeles, California 90095; email: txia@ucla.edu

[2]Asthma and Allergic Diseases Cooperative Research Centers, University of California, Los Angeles, California 90095; email: nli@mednet.ucla.edu

[3]The Southern California Particle Center, University of California, Los Angeles, California 90095

[4]California NanoSystems Institute, University of California, Los Angeles, California 90095; email: anel@mednet.ucla.edu

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Abstract

Although mankind stands to obtain great benefit from nanotechnology, it is important to consider the potential health impacts of nanomaterials (NMs). This consideration has launched the field of nanotoxicology, which is charged with assessing toxicological potential as well as promoting safe design and use of NMs. Although no human ailments have been ascribed to NMs thus far, early experimental studies indicate that NMs could initiate adverse biological responses that can lead to toxicological outcomes. One of the principal mechanisms is the generation of reactive oxygen species and oxidant injury. Because oxidant injury is also a major mechanism by which ambient ultrafine particles can induce adverse health effects, it is useful to consider the lessons learned from studying ambient particles. This review discusses the toxicological potential of NMs by comparing the possible injury mechanisms and adverse health effects of engineered and ambient ultrafine particles.

INTRODUCTION

Nanomaterials (NMs): materials ranging from 1 to 100 nm in at least one dimension

NP: nanoparticle

UFP: (ambient) ultrafine particle

DEP: diesel exhaust particle

Nanotoxicology: study of toxicity induced by nanomaterials

Reactive oxygen species (ROS): include oxygen species that contain one or more unpaired electrons, singlet oxygen, and organic or inorganic peroxides; highly reactive

Oxidative stress: a condition of decreased oxidant defense or increased oxidant production that exceeds biological system's ability to neutralize them

The sale of products utilizing nanotechnology could top an estimated $1 trillion per year by 2015. Nanotechnology is moving at a rapid global pace, often with a short time window between the actual date of discovery and the point at which new inventions are commercialized. According to the Nanotechnology Consumer Products Inventory, more than 600 self-claimed nanotechnology products are currently being produced by 322 companies in 20 countries (47). Nanomaterials (NMs) with novel physicochemical properties are often being used to improve the functionality of new commercial products. Examples of such products are sunscreens, paints, cosmetics, clothing, building materials, electronics, and personal care products. Although these products benefit the users and the economy, increased exposure of nanotechnology researchers, workers, and consumers to potentially hazardous materials could cause adverse health effects. Workers involved in manufacturing, shipping, or handling of nanoparticles (NPs) are probably already being exposed to some of the materials that are produced in bulk or powder form. In addition to exposing workers through occupational encounters, the use of NM for drug therapy, imaging, and gene delivery is also increasing personal exposure. Although these exposures may raise some concern, little is known about the toxic potential of these NMs on human health. Although much still needs to be learned about the toxicity of engineered NMs, we are fortunate to start with a clean slate such that there are no documented incidences of a human or occupational disease due to an engineered NP exposure.

Although clinical toxicity by engineered NM has not been documented thus far, the literature on particles and fibers, including ultrafine particles (UFPs), diesel exhaust particles (DEPs), quartz, and asbestos (51, 58), indicates a history of adverse health effects. It is possible therefore that engineered NPs and fibers may pose similar hazards: The novel physicochemical properties of these engineered materials may introduce new mechanisms of injury and toxicological paradigms. Much could be learned, however, from the study of inadvertent particle and fiber exposures. In fact, from an occupational perspective, the study of nanoparticle toxicity is a mature science that has led to considerable insight into particle and fiber injury.

AMBIENT UFP EFFECTS AND IMPLICATIONS FOR ENGINEERED NPs

UFPs are an example of incidental particles that, when inhaled, can lead to adverse health outcomes. These particles have an aerodynamic diameter of <100 nm and are therefore true NPs. Their small size distinguishes them from larger coarse (aerodynamic diameter <10 μm, PM_{10}) and fine (aerodynamic diameter <2.5 μm, $PM_{2.5}$) air pollution particles. These particles could be derived from the fossil fuel combustion process, e.g. diesel exhaust particles (DEP), or through the condensation of semivolatile substances. Other examples of respirable nanoscale particles that can induce toxicological effects are quartz and mineral dust particles. The development of the Versatile Aerosol Concentration Enrichment Systems (VACES) in the Southern California Particle Center has allowed investigators to collect concentrated ambient particles (CAPs) of various size ranges, including UFPs (36, 75). Using this technology, we have conducted comparative toxicological studies on coarse, fine, and ultrafine particles obtained from the Los Angeles basin. This approach has also enhanced research on the mechanisms of PM injury in animal disease models. This research suggests that, among different particle sizes, UFPs are potentially the most dangerous owing to their small size, deep penetration, large surface area/volume ratio, high content of redox cycling organic chemicals, deep penetration, and high rates of retention in the lung (36, 50). Moreover, from a mechanistic perspective, we have demonstrated that the capability of UFPs to generate reactive oxygen species (ROS) and oxidative stress is a

Table 1 Comparison of ambient ultrafine particles (UFPs) and nanoparticles (NPs)[a]

Particle types	UFPs	NPs
Source	Incidental (combustion)	Engineered (controlled synthesis)
Surface area/volume	High	High
Uniformity	Low	High (size, shape, functionality)
Organic chemical content	High	Low
Metal impurities	High	Varies
ROS generation	Yes	Varies
Exposure route	Inhalation	Inhalation, skin, ingestion, injection
Adverse health effects	Yes	Unknown

[a]Abbreviation: ROS, reactive oxygen species.

key injury mechanism that relates to proinflammatory and proatherogenic effects in the respiratory and cardiovascular tracks, respectively (13, 36, 50, 58, 79).

Some of these principles may be applicable to engineered NPs. However, UFPs and engineered NPs differ in many aspects such as sources, composition, homo- or heterogeneity, size distribution, oxidant potential, and potential routes of exposure (**Table 1**). The potential adverse health effects induced by engineered NPs are still largely unknown.

AMBIENT UFPs GENERATE ADVERSE PULMONARY AND RESPIRATORY EFFECTS

Epidemiological studies show that a sudden surge in the level of particulate matter (PM) can be linked to increased cardiorespiratory morbidity and mortality including asthma, chronic obstructive pulmonary disease (COPD), and atherosclerosis (4, 12, 64). Although several mechanisms may explain these adverse health outcomes, a number of in vitro, in vivo, and human experiments have shown that cardiovascular and respiratory inflammation resulting from the induction of oxidative stress could play an important role in disease pathogenesis (13, 36, 50, 75, 79). DEP emissions and the condensation of semivolatile chemicals and sulfuric acid are major sources of ambient UFPs. DEPs have often been used as a model particulate pollutant to study the injury by which ambient UFPs may contribute to pulmonary inflammation and asthma. The experimental evidence collected to date demonstrates that ROS generation and the induction of oxidative stress by organic DEP chemicals may play a role in the proinflammatory and adjuvant effects of these particles in the lung (3, 21, 32, 37). Intratracheal DEP instillation increases polymorphonulear cell infiltration, mucus production, NO release, and airway hyperreactivity (AHR) in mice, all of which play important roles in the pathogenesis of asthma (20, 25, 40). DEP and UFP are capable of inducing allergic inflammation through an impact on the immune system. This reaction manifests as increased respiratory or nasal challenge responses to common environmental or experimental allergens in humans and mice (10, 11, 46). This is also known as an adjuvant effect and manifests as enhanced allergen-specific IgE and IL-4 production in the human nose during combined challenge with DEPs and the ragweed allergen (11). Intranasal instillation of DEP also increased the expression of several chemokines, including RANTES, MIP-1α, and MCP-1 (10). In animal studies, DEPs enhanced ovalbumin (OVA)-induced eosinophilic airway inflammation, OVA-specific IgG1 and IgE production, goblet cell proliferation, and local expression of Th2 cytokines and chemokines (80). Investigators have reported similar results in animals receiving intratracheal instillation of the dust mite allergen, Der f, in the presence of DEPs (26, 63).

POTENTIAL ADVERSE PULMONARY AND RESPIRATORY EFFECTS OF ENGINEERED NPs

Although the synthesis of engineered NMs often takes place under controlled gas-phase conditions, several particle types such as TiO_2, carbon black, zinc oxide, and other metal oxides are produced in powder form. Thus these particles could be stirred up in the air during packaging, handling, or accidental spills. Therefore, some categories of engineered NPs could be inhaled. Although there are no examples to date of respiratory pathology in humans due to the inhalation of engineered NPs, cellular and animal experiments have shown that some types of NPs are capable of generating proinflammatory and prooxidative effects that could lead to respiratory pathology (8, 14, 51, 58).

For example, subacute exposure of C57B1/6 mice to 2–5 nm TiO_2 NPs caused a moderate but significant inflammatory response in the lung within the first two weeks of exposure, after which the inflammation resolved without permanent damage (19). The same particle type has also induced pulmonary emphysema, macrophage accumulation, alveolar septal disruption, type II pneumocyte hyperplasia, and epithelial cell apoptosis in Institute of Cancer Research (ICR) mice (6). Ultrafine colloidal silica particles (UFCSi) induce more severe pulmonary inflammation after intratracheal instillation in ICR mice (29). One reason for the injury and inflammation could relate to the large surface area (29). Particle surface area is a more appropriate dose metric than particle mass or particle number when evaluating the dose-response relationships in the lung (58). This notion was confirmed when comparing fine with ultrafine particles for a number of material types, including TiO_2 (14, 58). However, the issue of NP toxicity goes further than just surface area and also needs to consider the surface reactivity (65, 74). A study by Warheit et al. showed that while ultrafine TiO_2 particles (P25) with an 80/20 anatase/rutile content induced sustained inflammation in the lung, the less reactive rutile form of the same particle exerted short-term pulmonary effects (65, 74).

In addition to the proinflammatory effects, NPs may also exacerbate existing lung diseases (17, 27). Inoue et al. (27) have demonstrated that intratracheal administration of 14 nm and 56 nm carbon black NPs induced slight lung inflammation and significant pulmonary edema compared with the phosphate buffered saline (PBS)-treated mice. However, when 14-nm carbon black NPs were coadministered with a bacterial endotoxin, these particles intensively aggravated lipopolysaccharide (LPS) -induced pulmonary inflammation (17, 27). Intratracheal instillation of Cabosil, a commercially available NP of amorphous SiO_2, caused significantly increased inflammatory changes in rat lungs compromised by bleomycin (17). These data suggest that engineered NPs could potentiate the effects of other inhaled stimuli (27).

THE PULMONARY TOXICITY OF CATIONIC NPs: THE ARDYSTIL SYNDROME

Cationic spray paint particles induced pulmonary toxicity in an occupational setting in Spain and Algeria (22). This toxicity, also known as the Ardystil syndrome, resulted in seven deaths from acute pulmonary edema in the 1990s (59). The affected subjects developed an array of complaints including upper respiratory complaints, nose bleeds, coughing, and bronchial hyperreactivity. Moreover, some of the survivors eventually developed pulmonary fibrosis. Although it was difficult to pinpoint the exact toxicological component in the spray paint, epidemiological and toxicological studies have implicated a polycationic paint component as the most likely culprit. Nemery et al. (23) performed in vitro and in vivo studies demonstrating that polycationic paint components could exert toxic effects in cells and animals. These toxic effects could be neutralized by polyanions, confirming the presence of potentially toxic cations (22). These results serve as a warning that engineered NPs that carry a positive surface charge may cause similar

health hazards. This notion is further bolstered by the increased in vitro cytotoxicity and in vivo pulmonary toxicity of cationic polystyrene nanospheres compared with anionic and plain ones (55, 78). Cationic polystyrene nanospheres can lead to toxicity in macrophages and epithelial cells, whereas anionic and nondecorated nanospheres are nontoxic (78). Intratracheally instilling the same nanospheres in mice resulted in increased neutraphil cell counts, protein content, and lactate dehydrogenase levels in the bronchoalveolar lavage (BAL) fluid (78).

THE PULMONARY TOXICITY OF METAL OXIDE NPs: METAL FUME FEVER

Metal fume fever (MFF) is a clinical syndrome in welders resulting from the inhalation of highly concentrated metal oxide particles, most commonly zinc oxide. Although the inhaled particles are likely to be in the fine or ultrafine range, these are not true engineered NPs. This syndrome is characterized by the sudden onset of a high fever, cough, headache, nausea, and vomiting. In spite of the dramatic symptoms, there is often no radiographic evidence of acute or permanent pulmonary damage (16). Although the pathogenesis of MFF is improperly understood, one suggestion is that the large-scale release of cytokines by pulmonary macrophages and other cell types is responsible for the onset this illness. Some studies have indicated that the inhalation of ZnO particles and metal fumes results in significant increases in polymorphonuclear leukocytes and lymphocytes in the BAL fluid, in parallel with increased TNF, IL-6, and IL-8 production (33, 42, 62). ZnO particles also induce cytotoxicity and apoptosis in a variety of different mammalian cell types (28, 44), and a recent study in tissue culture cells has linked this outcome to the dissolution of the ZnO NPs and the ability to induce oxidative stress (77). It seems likely that toxic Zn^{2+} release could trigger a series of cellular effects that results in increased cytokine and chemokine production. However, the rapid dissolution of the particles may be responsible for the finding that the clinical symptoms are of short duration and do not lead to permanent respiratory damage (5).

THE POTENTIAL PULMONARY TOXICITY OF CARBON NANOTUBES: INTERSTITIAL PULMONARY FIBROSIS AND MESOTHELIOMA

Carbon nanotubes (CNTs) are engineered NMs that are produced as single-wall (SWNTs) or multiwall (MWNTs). Both types of materials exhibit unique electrical, mechanical, and thermal properties and have many applications in the electronics, computer, and biomedical fields and in the aerospace industry. From a toxicological perspective, when the length of these CNT strands are >20 μm and relatively stiff, the material may act as an indigestable fiber that cannot be destroyed in the phagosomes of macrophages. These CNT fibers could protrude through the cell wall and result in frustrated phagocytosis (15), which signifies that their indestructibility could lead to a pouring of oxygen radicals. When this process takes place in the pleural cavity or the peritoneum, it could result in chronic granulomatous inflammation, which could be the forerunner of mesothelioma. Two recent studies on MWNTs in mice suggest the carcinogenic potential of MWNTs in vivo (60, 72). One study showed that exposing the mesothelial lining of the peritoneal cavity to long indigestable MWNTs results in chronic peritoneal inflammation and formation of granulomas, whereas short MWNTs did not cause the same lesion (60). Another study demonstrates that long MWNT can induce peritoneal mesothelioma in $p53^{+/-}$ mice, which are very susceptible to developing the same malignancy in response to asbestos exposure (72). There is no evidence to date, however, to indicate that CNTs cause the same malignancy in humans. Although the cause of the malignancy in mice is uncertain, it is possible that the production of free oxygen radicals and chronic inflammation could lead to tumor initiation or promotion (31).

SWNTs are also capable of inducing interstitial pulmonary reactions in experimental animals. Shvedova et al. demonstrated that pharyngeal aspiration of SWNTs elicited unusual pulmonary effects in C57BL/6 mice. This abnormality manifests as acute inflammation that leads to progressive interstitial fibrosis (69). The progressive fibrosis presented as two distinct morphologies: (*a*) SWNT-induced granulomas that were associated with hypertrophied epithelial cells surrounding SWNT aggregates, and (*b*) diffuse interstitial fibrosis and alveolar wall thickening associated with dispersed SWNT (70). The latter effect is accompanied by the increased production of the fibrogenic cytokine transforming growth factor (TGF)-β1. A recent study by the same group compared inhalation to pharyngeal aspiration and found that the former route of exposure was more effective than aspiration in causing inflammation, oxidative stress, collagen deposition, and fibrosis (70). These reactions were accompanied by mutations of the *K-ras* gene locus in the lungs of these animals (70).

It is appropriate to ask, therefore, whether CNTs can be inhaled under real-life conditions in humans. CNTs are produced naturally in the soot created by the burning of various chemicals and compounds; these naturally occurring CNTs are highly irregular in size and are mixed with large amount of carbon soot (73). In contrast, commercial methods for engineered CNT production include high-pressure carbon monoxide (HiPco), chemical vapor deposition (CVD), and plasma-enhanced CVD. Most of these processes take place in a vacuum or in closed gas-phase environments. There is no evidence that these production methods lead to significant occupational exposures. CNT exposures could occur, however, if these materials are used as drug or gene carriers in the body. CNTs are also found in car tires, which, owing to wear and tear, could shed rubber particles that are inhaled and deposited in the lung. To date, however, no human pathology or disease has been ascribed to engineered CNTs and there are deliberate efforts to make use of the novel properties of CNTs for therapy or imaging purposes.

We have already discussed the toxicological potential of CNT in relation to material characteristics such as a high aspect ratio, biopersistance, and an indigestibility as well as the possiblity that this leads to frustrated phagocytosis and chronic inflammation. In vitro toxicity studies suggest that ROS production and the generation of oxidative stress could contribute to material toxicity (30, 68). One reason for this ROS generation is the presence of metal impurities, such as iron and copper, which are used as catalytic agents during the synthesis of the CNT (30, 68, 69), although extensive CNT purification eliminates these effects.

When properly functionalized, CNTs can be used as imaging agents that can be safely administered intravenously (66). No toxicity was observed over a month time period in some of these studies (66), which demonstrates that, if the material surface is properly designed, it is possible to render CNT biocompatible.

GENERATION OF ADVERSE CARDIOVASCULAR EFFECTS BY AMBIENT UFP

Epidemiological evidence indicates that PM exposure can induce morbid and fatal cardiovascular events such as atherosclerosis, which can lead to coronary artery disease, myocardial infarctions, and stroke (51, 58). Although this association has been most clearly documented for PM_{10} and more recently for $PM_{2.5}$, increasing experimental evidence indicates that ambient UFPs may pose an even greater health risk (36). Using our particle concentrator technology, we demonstrated that UFPs induce more atherosclerotic plaque in apoE knockout mice than does a mixed atmosphere of fine plus ultrafine particles (1). Although there are a number of possible explanations for this finding, we observed good correlation between the plaque development and the high content of organic chemicals on the UFP surface (1). Moreover, the increased rate of atherogenesis correlated

with increased evidence of systemic oxidative stress in UFP-exposed animals. Our hypothesis, therefore, is that UFP-induced oxidative stress could constitute the principal mechanism by which UFPs induce atherosclerotic plaque development. Because the atherosclerotic plaque is basically a chronic inflammatory lesion, there may be synergy between the PM-induced inflammation and the prooxidative and proinflammatory effects of oxidized low density lipoprotein (LDL). We have demonstrated through a genome-wide analysis that oxidized LDL components synergize with organic DEP chemicals in stimulating the expression of oxidative stress–responsive genes (18).

How does UFP inhalation lead to oxidative stress generation at a remote vascular site? One possibility is that inhaled particles may release organic chemicals and transition metals from the lung to the systemic circulation. Another is that pulmonary inflammation could lead to the release of ROS, cytokines, and chemokines to the systemic circulation. The third possibility is that UFPs could gain access to the systemic circulation by directly penetrating the alveolar/capillary barrier in the lung (54). Indeed, some reports in the literature show the systemic translocation of ^{99m}Tc-labeled ultrafine carbon particles (54) or albumin nanocolloid particles of <80 nm (56). However, a detailed mechanism for the inhaled particle translocation remains to be determined (67).

EXPERIMENTAL STUDIES LOOKING AT THE EFFECTS OF ENGINEERED NPs ON THE CARDIOVASCULAR SYSTEM

Although no human data, to date, show adverse cardiovascular effects of engineered NMs, the use of these materials for imaging and therapeutic purposes could pose a health risk. Limited experimental evidence has emerged showing that NMs could produce adverse cardiovascular impacts. Data include reports that engineered NPs may penetrate the pulmonary epithelial cell barrier, enter the systemic circulation, and gain access to the cardiovascular system (57, 58). Carbon NMs such as MWNT, SWNT, and carbon black NPs induced human platelet aggregation in vitro and promoted arterial thrombosis in rats (61). One explanation is the ability of NMs to increase GPIIb/IIIa expression on platelets, in addition to activating signaling pathways involved in platelet aggregation (61). Nemmar et al. (53) reported similar prothrombotic effects for CNTs. Intratracheal instillation of MWNTs in mice triggers mild lung inflammation, which can induce secondary platelet activation in the systemic circulation (53). Platelet activation recruits more leukocytes to form platelet-leukocyte conjugates that produce secondary procoagulant effects by releasing the tissue factor (53). In contrast to the prothrombotic effects of CNTs, Zhu et al. (81) reported that intratracheal instillation of Fe_2O_3 NPs lengthened blood prothrombin time and activated partial thromboplastin times in rats, which suggests that nanoparticles could also exert anticoagulant effects.

CNTs may promote atherosclerosis (38). A single intrapharyngeal SWNT instillation in mice induced oxidative stress in the lung, heart, and aorta (38). This result was reflected by a reduced glutathione-to-oxidized-glutathione ratio (GSH/GSSG) and increased protein carbonyls in the aorta (38). Chronic exposure of ApoE$^{-/-}$ transgenic mice to SWNT, while being fed an atherogenic diet, led to accelerated atherosclerotic plaque formation as well as mitochondrial DNA damage (38). All considered, while these preliminary findings indicate that engineered NM may induce cardiovascular effects, there are no documented incidences of human cardiovascular disease due to engineered NM exposure.

HOW DO WE APPROACH THE TOXICITY OF POTENTIALLY HAZARDOUS NMs, AND WHAT CAN BE DONE TO PREVENT THE GENERATION OF ADVERSE HEALTH EFFECTS IN WORKERS AND CONSUMERS?

The experimental effects of some NMs show that they can induce adverse biological

responses at cellular, subcellular, membrane, protein, tissue, and organ levels. The potential for biological injury lies in the novel physicochemical properties of NMs as they approach the sub-100-nm length scale. NMs have a much larger surface-area-to-volume ratio than do bulk materials, which means that an increased number of atoms are exposed at the material's surface. Quantum effects dominate at this length scale. As a result, increased surface reactivity of NMs could facilitate interactions with biological molecules such as DNA, proteins, and membranes, which also function as nanoscale structures. The interactions taking place at the nano-bio interface could have many consequences, including, but not limited to, oxidant injury, conformational change, membrane permeability changes, mutational alteration, signaling effects, ionic exchanges, biocatalytic changes, enzyme failure, and new epitope exposure in the proteins.

The most important NM properties involved in nano-bio interactions include size, shape, purity, surface area, charge, hydrophobicity, state of aggregation, crystallinity, electronic state, and potential to generate ROS. These properties may be related to biological outcomes according to a number of structure-activity flow diagrams, one example of which is shown in **Figure 1** (45). The integration of these modules may determine the material's biocompatibility or toxicity. Each type of material could establish its own structure-activity diagram, which for a given biological substrate means more or fewer interactions based on the material's physical-chemical characteristics. The relationship between material characteristics and toxicological outcomes can also be used for the safe design of nanomaterials. Improvements could include changing or adapting physicochemical characteristics that decrease cellular uptake or bio-availability, prevent spread, or decrease injurious biocatalytic effects. One example of how these improvements can be accomplished is by NP surface coating with polymers, ligands, and detergents that provide steric hindrance (or block access) to the particle surface.

A number of the structure-activity relationships can be used to screen for NP toxicity. Examples are NP interactions with the cell membrane, cellular uptake, and subcellular localization (51, 58). Key NM characteristics that promote particle wrapping at the cell membrane and cellular uptake include size, shape, charge, aggregation status, surface roughness, hydrophobicity, and the presence of surface coatings or surface ligands (51, 58). The surface characteristics can lead to specific as well as nonspecific binding interactions with the membrane. Receptor-mediated endocytosis is an example of the former, whereas the interaction of surface groups with charged phospholipid head groups or protein domains is an example of the latter (24, 34). Particle size is an important determinant of particle wrapping time and cellular uptake (7). Particle uptake can be studied by fluorescence and other imaging techniques, and wrapping time can be quantitatively expressed by a series of mathematical equations (7). Subcellular processing can also exert a crucial effect on the biological outcome (51, 78) (**Figure 2**). For instance, cationic charge can lead to lysosomal processing and cytotoxicity that is premised on the proton sponge hypothesis (78). This theory posits that extensive buffering by the cationic particle surface may lead to unchecked proton transport into the lysosome. This action could lead to excessive water influx, which, owing to the space constraints, could rupture the endosome. Our recent data show that the toxicity of cationic NPs (60 nm) in macrophages is premised on the proton sponge effect, whereas in epithelial cells the same particles induce a different pathway of toxicity (**Figure 2**). These data show that particle surface properties determine cellular uptake pathways, subcellular processing mechanisms, and cytotoxicity.

ROS and oxidative stress provide an important screening principle on the basis of consideration discussed earlier in this review. NM characteristics that promote or contribute to

ROS generation are (*a*) photoactivation effects, e.g., the formation of electron-hole pairs during UV exposure of TiO_2—this effect has been associated with the generation of oxidative stress and inflammation by TiO_2 (41, 71); (*b*) discontinuous crystal planes and material defects that lead to oxygen radical generation owing to the active electronic state of the material surface; (*c*) redox cycling, which contributes to ROS production. This can occur because of the presence of transition metals or redox cycling organic chemicals on the particle surface. UFPs, for example, contain organic compounds such as quinones, which can generate ROS through redox cycling. Moreover, transition metals can generate hydroxyl radicals through the Fenton reaction (52). The Fenton reaction is one of the mechanisms by which metal impurities on the CNT surface can induce ROS production (**Figure 3**). Finally, (*d*) particle dissolution (e.g., ZnO, CdSe, Cu) can produce free ions that are capable of inducing ROS generation and toxic effects in cells (1, 9, 43) (**Figure 3**). MFF may be an example of this toxicity.

USING THE OXIDATIVE STRESS PARADIGM AS A SCREENING ASSAY FOR NM TOXICITY

NM oxygen radical generation can result in cellular and tissue injury responses such as inflammation, apoptosis, necrosis, fibrosis, hypertrophy, metaplasia, and carcinogenesis (51). To use this paradigm as a screening procedure for NP toxicity, we have formulated the hierarchical oxidative stress model as an integrative method for testing a wide range of cellular injury responses. At the lowest level of oxidative stress (tier 1), the induction of antioxidant and protective responses is mediated by the transcription factor, Nrf2, which regulates the activation of the antioxidant response element in the promoters of phase II genes (35, 79). At higher levels of oxidative stress (tier 2), this protective response may yield to proinflammatory responses because ROS induces redox-sensitive signaling pathways such as the mitogen-activated protein kinase (MAPK) and nuclear factor-kappa B (NF-κB) cascades (79). At the highest level of oxidative stress (tier 3), a perturbation of mitochondrial inner membrane electron transfer and the open/close status of the permeability transition pore can trigger cellular apoptosis and cytotoxicity. This outcome is also known as toxic oxidative stress. Using this three-tier screening platform we compared a number of different materials as well as variations in the surface characteristics of a single material to discern between potentially hazardous or potentially safe NPs (76). Potentially safe NPs (such as carbon black and polystyrene) induced either no response or only a tier 1 response, whereas potentially hazardous NPs (such as metal oxides and ambient UFP) induce proinflammatory (tier 2) or cytotoxic (tier 3) effects. We have also shown for ambient air pollution particles that one can link the hierarchical oxidative stress paradigm to the in vivo outcomes in animal disease models, as reviewed above (2, 18).

Using the oxidative stress paradigm constitutes one of the first examples that could become a predictive screening paradigm for NM toxicity. By predictive, we mean that an in vitro manifestation of toxicity translates into an in vivo toxicological outcome. One example is the link between the ability of UFP to induce proinflammatory effects in macrophages and epithelial cells and to enhance allergic inflammation in intact animals. The emphasis on predictive screening paradigms is becoming more important as the number of new NMs are expanding because previous experience in the chemical industry has shown that it is not cost-effective or logistically feasible to test all the compounds (>50,000 industrial chemicals) by labor-intensive in vivo test procedures. Fewer than 1000 industrial chemicals have undergone toxicity testing because of the logistics and high costs of animal testing. It would clearly be more effective if we had predictive screening paradigms that could be used for high content or high-throughput screening of NMs and

then used this data to prioritize in vivo animal test schedules. Recent development in high-throughput toxicity screening (HTS) procedures makes predictive in vitro testing possible with fewer associated costs and shorter timeframes. The National Research Council of the U.S. National Academy of Sciences (NAS) recently opined that toxicological testing in the twenty-first century should undergo a paradigm shift from a predominant observational science in animals to a target-specific and predictive in vitro science that utilizes mechanisms of injury and toxicological pathways to guide conductance of in vivo studies (48, 49). This opinion is also compatible with the increased public demand and regulatory demand to reduce animal use for toxicological screening, e.g., the recent European Union REACH legislation. This legislation requires the development of extensive toxicological testing by ex vivo approaches. Predictive screening models based on quantitative structure-activity relationships of NMs will likely become progressively more important as the number of new nanoproducts increases.

CONCLUSIONS

Rapid development of nanotechnology and commercialization of nanoproducts increases the risk of human exposure to engineered NMs. It is imperative to establish a scientific basis for understanding the toxic potential of these materials. Studies of the adverse health effects of ambient air pollution particles, asbestos, and quartz provide considerable insight into potential NP and nanofiber injury. One of the principal mechanisms of toxicity induced by these particles/fibers is the generation of reactive oxygen species and oxidant injury. It is likely that nanoparticles with novel physicochemical properties may introduce new mechanisms of injury. Therefore, it is important to understand the interactions happening at the nano-bio interface and identify toxicological pathways and mechanisms of injury. This understanding can be used to develop predictive screening paradigms for NM toxicity. Achieving this goal requires a paradigm shift from a predominant observational science in animals to a screening paradigm that could be used to prioritize in vivo testing.

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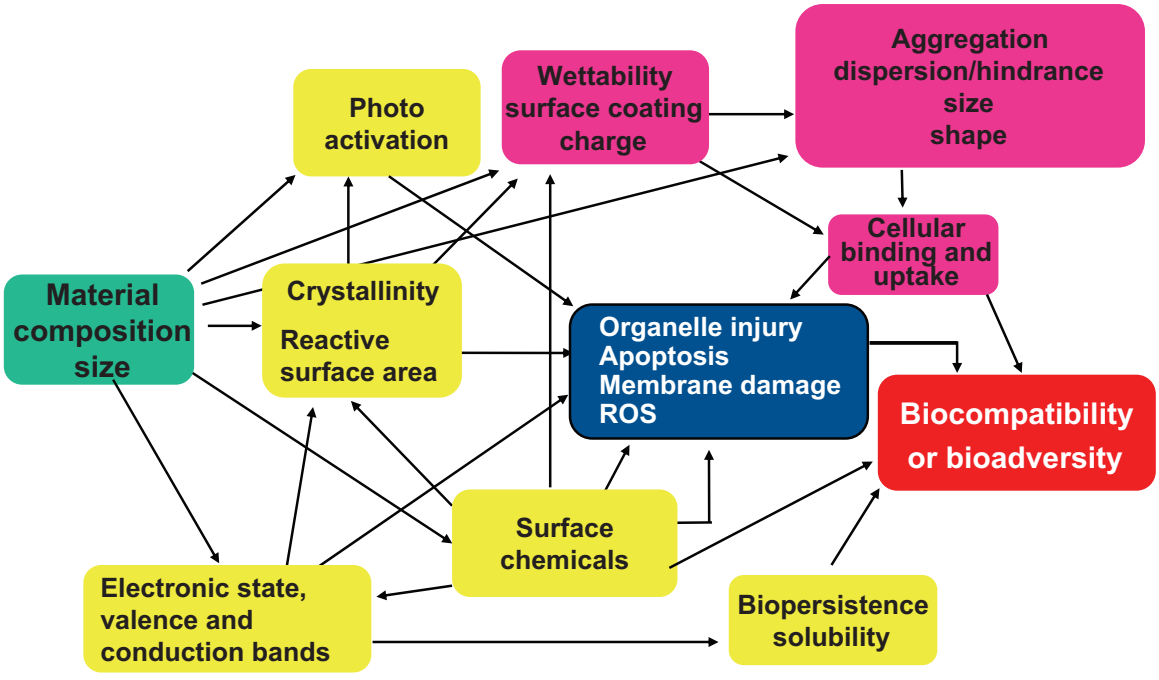

Figure 1

Postulated quantitative structure-activity relationship (QSARs) linking NM properties to biological outcomes. The physicochemical characteristics of NPs can be divided into overlapping modules as one set of material characteristics that determine surface reactivity, another set of material characteristics that determine the cellular uptake and subcellular localization, and a third set of material characteristics that determine the interaction with specific cellular compartments or processes. The integration of these modules may determine the biocompatibility or toxicity of the material (modified from 45).

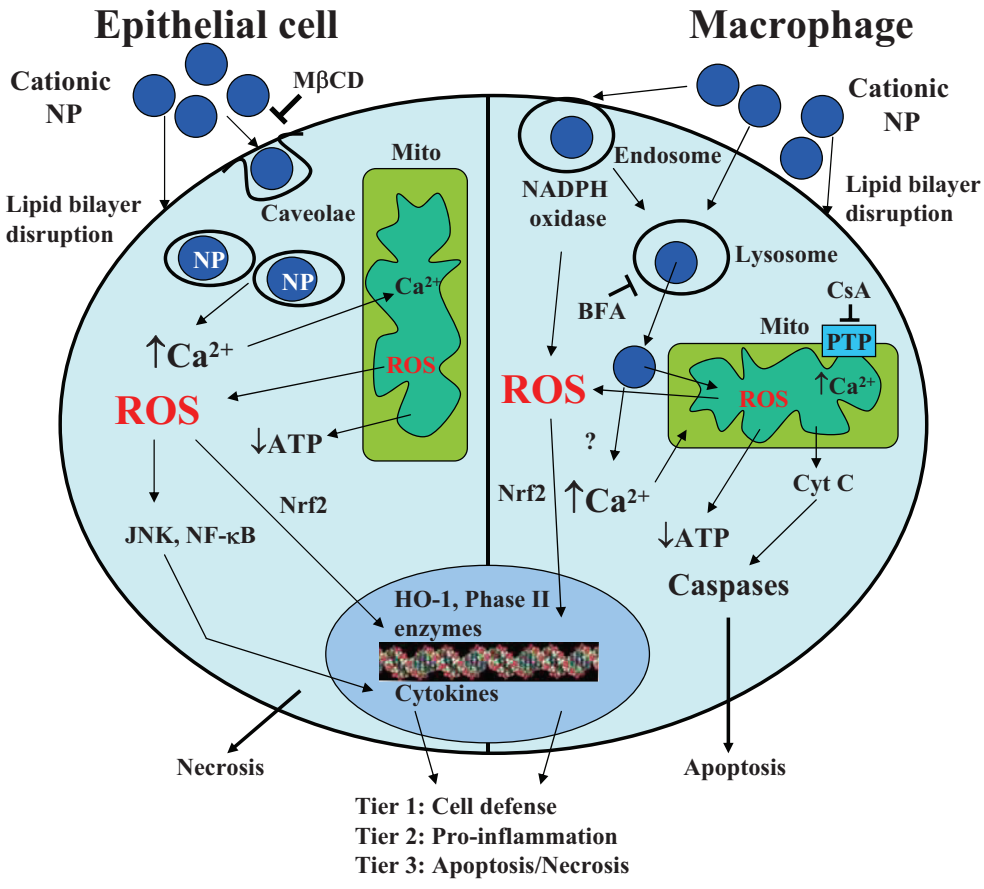

Figure 2

Comparison of the mechanisms of cell death induced by cationic NPs. NMs are taken up into cells via endocytosis. In macrophages, particles are taken up into phagosomes, and the formation of functional nicotinamide adenine dinucleotide phosphate (NADPH) oxidase produces ROS. Cationic particles enter a LAMP-1 positive lysosomal compartment in macrophages and induce lysosomal rupture via the proton sponge effect. A proton pump inhibitor, BFA, interferes in this pathway. Subsequent deposition of the particles in the cytosol induces an increase in mitochondrial Ca^{2+} uptake, oxidative stress, PT pore opening, and apoptosis that could be suppressed bycyclosporin A (CsA). In contrast, cationic NPs are taken up into epithelial cells via caveolae, and the uptake and toxicity could be inhibited by MβCD through cholesterol extraction from the plasma membrane. Cationic NPs also induce Ca^{2+} increase, oxidative stress, and mitochondrial damage; however, epithelial cells undergo necrosis. Cells under oxidative stress can typically induce tiered responses including cell defense (tier 1), proinflammation (tier 2), and mitochondria-mediated cell death (tier 3).

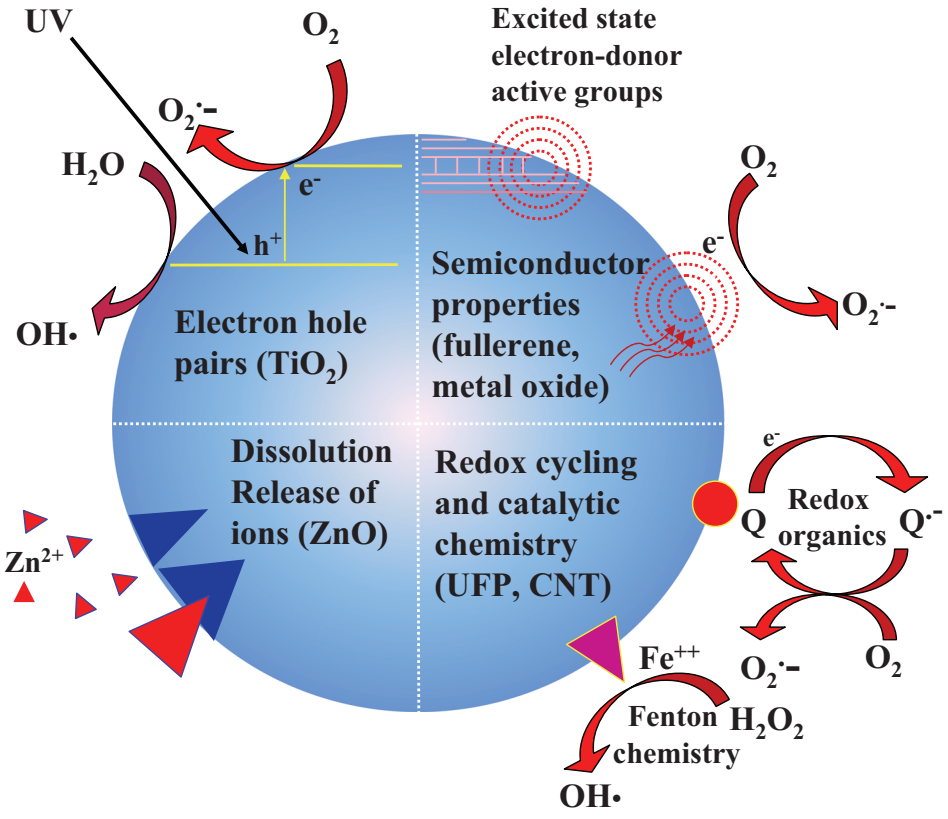

Figure 3

NM surface properties that generate ROS. The valence and conductance bands of semiconductor NMs can generate electronic states that lead to the formation of $O_2^{\cdot-}$, which through dismutation or Fenton chemistry can generate additional ROS. Also, photoactivation of TiO_2 could generate electron hole pairs that generate $O_2^{\cdot-}$ and OH· radicals. Transition metals and redox cycling of organic chemicals on the particle surface can also generate ROS. Dissolution of the particle surface by releasing metal ions could be particularly relevant to ZnO particle toxicity. These dissolution characteristics could vary with the free surface energy of the particles as well as the pH of the environment or the cell. Adapted with permission from Reference 39.

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